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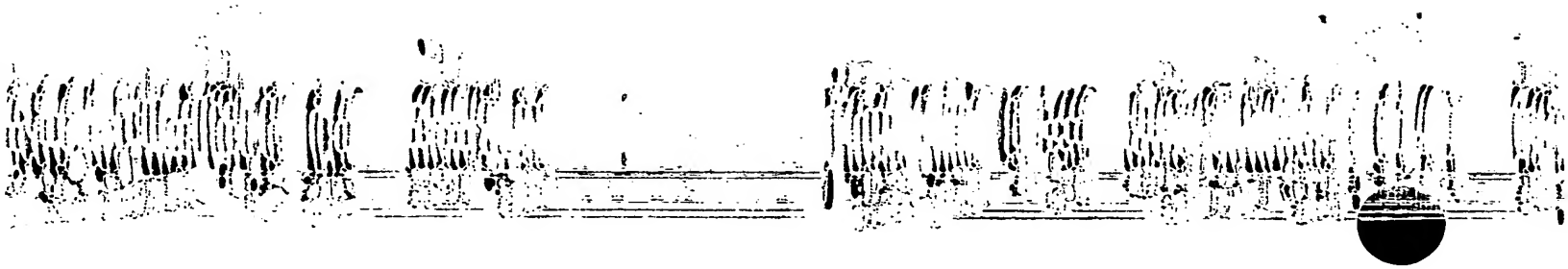

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The present invention relates to a method for detecting rodent infestations.

Urban environments provide suitable conditions for certain species of rodents, particularly Norway rats and house mice to proliferate. An essential feature of successful human urban environments, is the efficient partitioning of certain features of the environment, such as waste disposal and food preparation. Mice and rats breach the partitions between such elements and consequently pose a health hazard. Rodent urine *per se* presents an additional health hazard because of the potential presence of *Leptospira spp.*, which can enter the blood through skin cuts causing the potentially fatal Weil's disease. In addition, they often impose a significant economic cost through the potential destruction of materials such as wall and roof insulation, electrical wiring and packaging, and the spoiling of foodstuffs. In order to devise and target efficient pest control strategies it is essential to have a reliable and sensitive method of identifying the presence and location of rodents in the environment and of identifying the species concerned.

Conventional methods of detection rely on an infestation reaching large enough proportions to show physical signs, the presence of faeces and signs of gnawing damage are the most commonly used. Whilst such signs can be reliable, they usually identify an infestation that is well established, which will be difficult to eradicate and could already have caused much damage. Small numbers of rodents will not always leave such obvious signs in visible sites. Droppings from mice are often difficult to spot, while those from rats are usually located in small numbers of latrine sites. Therefore, whilst such methods can be reliable for detecting large scale infestations, they are usually inadequate for detecting the presence of rodents at a low level. Furthermore the removal of physical indications will mask the presence of an infestation and it is therefore possible for a person to conceal an infestation by clearing droppings etc.

Given the public [illegible] associated with [illegible] and [illegible] they inhabit, detection of low levels of infestation and/or the detection of infestation which have been disguised in sensitive areas (such as kitchens and hospital wards) is desirable. It is therefore an aim of the present invention to provide a method of detecting rodent infestations which obviates or mitigates the disadvantages associated with prior art methods.

According to the present invention there is provided a method of detecting a rodent infestation comprising detecting for the presence of urinary proteins from rodents on a surface on which urinary proteins have been deposited.

By "urinary protein from rodents" we mean the proteins that are naturally found in the urine of healthy rodents. Urine from rodents such as the house mouse (*Mus domesticus)* possesses an almost unique chemical property in that it contains a high concentration of protein. Such a phenomenon is known as an obligate proteinuria. In contrast, proteinuria in humans invariably occurs as a consequence of kidney dysfunction whereby damaged kidneys effectively "leaks" serum proteins into the urine. In humans, the content of the proteinuria reflects that of the serum, i.e. the most abundant serum protein, albumin, is found in the urine whereas the obligate proteinuria of the house mouse, and other members of the *Mus* genus, has a distinct protein composition.

Rodents such as mice are nocturnal, burrowing animals which, unlike humans, have a highly developed sense of smell. Olfactory communication in mice mediates many functions, including kin recognition, orientation, mate selection, reproductive priming, territorial advertisement and competitive interactions. Many olfactory signals used by mice are contained in the urine. Such signals can affect both their behaviour and physiology. As a consequence, house mice deposit their urine as scent marks, deliberately distributing their urine as small streaks and spots on all surfaces they pass over in their environment. Both male and female mice of all ages (excluding nestlings) deposit their urine in this way, though socially dominant adult males deposit more marks than other classes. House mice are stimulated to mark any

colonising a new area and will mark any new (unmarked) objects appearing in their territory. The presence or odour of any potential competitors in their territory usually stimulates a very strong counter-marking response from the territory owner and mice will continually remark key sites such as territory borders, entrances to nest sites or around food sites, often resulting in the build up of small visible mounds of dried urine, termed urine posts. Similar marking is shown by free living wild house mice.

An analysis of normal human and laboratory mouse urine (e.g. by SDS-PAGE) demonstrates that the mouse proteinuria is comprised predominantly of a set of closely related proteins, termed Major Urinary Proteins (MUPs). Accordingly when the method of the invention is used to detect for the presence of mice it is preferred that the urinary protein detected is MUP.

MUPs are small, acidic proteins (Mr = 18.5KDa, pI ~ 4.5) with a distinctive three dimensional structure which have been extensively characterised (e.g. see Robertson *et al*, 1996. Biochem J. 316 p265-272). The polypeptide chain forms a number of strands (β strands), which are arranged in a cross hatched barrel conformation (β-barrel), around a central hydrophobic pocket or calyx. Proteins which possess this distinctive three dimensional structure have been classified into one superfamily, the lipocalins. A large number of functions have been assigned to members of the lipocalin superfamily, many of which involve transport of hydrophobic ligands in the central cavity. In MUPs, this hydrophobic cavity is home to at least two small organic ligands, 2-*sec*-butyl-4,5-dihydrothiazole (thiazole) and 3,4-dehydro-*exo*-brevicomin (brevicomin). Both these ligands are mouse olfactory signals or pheromones. Although the precise function of MUPs is not known, they have been shown to prolong short lived olfactory signals and can act as an olfactory signal in its own right. Urine contains a high concentration of MUPs (usually around 15mg/ml in adult male BALB/c mice), which represents a significant loss of protein (and the metabolic energy invested in its manufacture) from the mouse. Consequently, MUP is thought to have a central role in mouse olfactory communication.

As stated above, MUPs are a mixture of closely related proteins, produced [redacted] a number of closely related genes. In inbred mouse urine, 15 individual MUPs have been identified by iso-electric focusing. The difference between individual MUPs is the result of small changes in the amino acid sequence of the polypeptide chain, which have little effect on the three dimensional structure. MUPs are produced by a multigene family, consisting of approximately 35 members, located on chromosome 4. Not all of these genes produce MUPs, only 15 are thought to encode proteins, whilst the remainder are silent or pseudogenes. The genes that produce urinary MUPs are expressed in the liver, from where the mature proteins are rapidly secreted into the blood and efficiently transferred from the blood into the urine by the kidney. Other MUP genes are expressed in a variety of other secretory tissues including the salivary glands. The expression level of MUP genes in the liver is under the control of a variety of hormones including testosterone, growth hormone and thyroxin. As a consequence, MUP expression shows sexual dimorphism, the concentration of MUPs in adult male urine is up to fifteen times greater than that of females or immature males. Therefore the method of the invention may be used to differentiate between the presence of male and female rodents.

Using anion exchange chromatography and electrospray ionisation mass spectrometry, we have identified a total of 9 distinct MUPs from two strains of inbred laboratory mice. The amino acid sequence of a number of MUPs has been inferred from cDNA sequences (DNA copied from mRNA) and the presence of MUPs with such sequences in urine confirmed from their molecular mass. The amino acid sequences of MUPs show a high degree of homology. Examples of known MUP amino acid sequences include Genbank database sequences with accession numbers X00907, M16355 and M16356 (derived from BALB/C mice) and also X00908 (derived from C57BL/6 mice).

Rats also possess a urinary protein, termed α2u globulin. Accordingly when the method of the invention is used to detect for the presence of rats it is preferred that the urinary protein detected is α2u globulin.

α2u globulin is also produced by a multigene family and exhibits sexual dimorphism. A comparison of a MUP and an α2u globulin amino acid sequence shows that they have only a limited homology (~30%). The three dimensional structure of α2u globulin is however very similar to MUP. Although the concentration of α2u globulin in rat urine is lower than MUPs in mouse urine, it is a significant output, again suggesting it has a central function in olfactory communication. The urine deposition patterns of rats are very similar to those of house mice (above). Rats will deposit streaks and spots of urine on any unmarked surfaces in the home area and respond to the presence of competitor rats of their odours with a strong counter-marking response.

It will be appreciated that different species of rodents may express different proteins in the urine. Such proteins may also be detected according to the invention.

Examples of other proteins found in rodent urine which may be detected according to the method of the invention include serum albumin and urinary pepsinogen.

In the light of the above we have developed methods for detecting rodent urinary proteins. Preferably the proteins are detected on a surface over which a rodent has putatively travelled and on which they have left their "mark".

The method of the present invention, as described in more detail below has been developed to provide a sensitive means of detecting rodents (and optionally distinguishing between species), in situations where established methods would fail. As illustrated in the example the method is sensitive enough to detect the footprint of a single rodent and therefore represents a significant improvement in the art.

Any suitable assay method may be used for detecting urinary proteins according to the method of the invention. However, it is preferred that rodent infestations are detected by utilising antibodies raised against urinary proteins from

rodent infestation immunoassay which is for the presence of proteins sample taken from an area which putatively has a rodent infestation.

Antibodies raised to MUPs and α2u globulins provide a means to detect extremely low levels of both proteins. As both proteins share only limited sequence homology, identification of the infesting species is also possible with antibodies which react specifically with each protein. Therefore according to one embodiment of the invention a plurality of antibodies may be used each of which is raised against a epitope on a urinary protein which is specific to a particular rodent species.

When an immunoassay is used for detecting the urinary proteins, binding of an antibody to a urinary protein must lead to the development of a measurable signal. Any conventional assay method may be used (e.g. radio labelled antibodies or ELISA systems).

A preferred assay method, which is described in more detail in the example, utilises a secondary antibody raised against a primary antibody raised against the urinary protein. This secondary antibody is linked to an enzyme which catalyses the formation of a product which produces a measurable signal in proportion to the amount of primary antibody complexed with urinary protein in or on a sample (e.g. a specific colour change). A preferred example of such an enzyme is alkaline phosphatase although it will be appreciated that various alternatives exist (including commercially available enzyme-linked secondary antibodies).

Rodent infestations may be detected according to the method of the present invention by taking samples from a surface on which the proteins have been deposited or over which a rodent has putatively travelled. The samples may be taken by a field operative as swabs and may be returned to a central laboratory to be tested for the presence of urinary proteins (e.g. by immunoassay). Alternatively an operative may have a portable kit or detector which may be used to assay for the presence of urinary proteins whilst the operative is still on site.

the surface on which the proteins have been deposited or over which a rodent has putatively travelled. This substratum may then be removed and the urinary proteins deposited on the substratum detected. Such substrata may be placed in strategic areas of a room, warehouse or similar area where a pest controller may suspect rodents will travel. After a given amount of time (e.g. over night) the substrata may be collected and tested for the presence of urinary proteins left as "marks" by passing rodents.

The substratum may be any material on which the proteins may be collected. The substratum may be required to vary in flexibility or have differing absorptive properties depending upon the surface and environment in which it is being placed. A preferred substratum is a sheet or tile. A most preferred tile or sheet is made of perspex (or similar material) which may be overlaid with a nitrocellulose membrane. Urine deposited by rodents are bound to the membrane and may be detected using a suitable assay for the urinary proteins

According to a preferred embodiment of the present invention urinary proteins from mice (MUPs) may be detected on nitrocellulose membranes which have been collected from an area which is putatively infested with rodents. Urine deposited by mice may be immobilised on the nitrocellulose membrane, either by direct deposition or by transfer from an element (e.g. a tile of the environment. Immobilised proteins are initially allowed to react with rabbit serum containing antibodies to MUPs, such antibodies are produced by the rabbit in response to a series of injections of purified MUPs. Unbound antibodies and serum proteins are then removed from the membrane by washing with a buffer solution. The membrane then undergoes a second reaction with a solution of antibodies raised in a goat, to rabbit antibodies. Such goat antibodies are additionally conjugated to the enzyme alkaline phosphatase. Unbound goat antibodies are again removed by washing. Finally, the membrane is developed in a solution containing a chromogenic substrate of alkaline phosphatase. In summary; areas of the membrane containing urinary proteins react with the specific antibodies in the rabbit serum, such bound antibodies react in turn with the goat antibody conjugate, which in turn reacts with the chromogenic substrate to produce a

such as this routinely detect microgram amounts of immobilised protein.

The present invention will now be described, by way of example, with reference to the accompanying drawings in which:

Figure 1 represents the immunoreactivity of rabbit anti MUP serum to mouse urine, liver and serum;

Figure 2 illustrates the MUP status in a variety of wild mice;

Figure 3 illustrates urine deposition by rodents visualised by immunochemistry;

Figure 4 illustrates urine marking behaviour in two individual mice using immunochemistry; and

Figure 5 illustrates the sensitivity of MUP immuno detection.

EXAMPLE

Experiments were performed to demonstrate the utility of the method of the invention for detecting rodent infestations. More specifically the urinary proteins found in the urine of house mice was detected in order to monitor for the presence of mouse infestations in human environments.

2. METHODS

2.1 Raising MUP specific antibodies

A 1ml aliquot of mouse urine, collected from male BALB/c laboratory mice by bladder massage, was desalted into 50mM MES buffer, pH5.0 using Sephadex G-25 spun columns. The desalted urinary proteins were then loaded onto a Mono-Q anion exchange column (Vt=1ml) which had previously been equilibrated with 20ml of the above MES buffer. After a brief wash, the column was developed with a linear NaC1 gradient of 0-200mM in 32ml. The column eluate was monitored for protein content by continuous UV spectroscopy at 280nm and collected as standard 0.5ml fractions. The elution profile resulting from this chromatography (centre plate, Fig. 2) contained four large peaks between elution volumes 22 and 34ml. The presence of MUPs in these peaks was confirmed by ESI/MS (outer plates, Fig. 2) prior to pooling them. The pooled protein was then diluted 1:3 with the above MES buffer and a 1ml aliquot was added to 1ml of Freund's complete adjuvent and the two solutions were emulsified by sonication. The emulsion was then injected subcutaneously into an albino New Zealand rabbit. The rabbit was injected twice more with the same preparation at monthly intervals, prior to being sacrificed and exanguinated.

2.2 Assessment of the presence and specificity of anti-MUP antibodies in rabbit serum

The presence of anti-MUP antibodies in the immunised rabbit serum, and their ability to react specifically with MUPs, was tested by performing western blot analysis on an extract of mouse liver proteins, mouse serum and mouse urine. All

other unrelated proteins.

A preparation of liver proteins was made by homogenising a fresh mouse liver in 20ml of water. Solid debris was removed by centrifugation resulting in a clear, pink solution. Serum was taken from mouse blood which had been allowed to clot overnight at 4°C. Urine was taken from groups of stock adult male BALB/c mice. These samples were then diluted 1:10, 1:20 and 1:500 respectively with SDS-PAGE sample buffer (1M tris/HC1, 7.5%(w/v) SDS, 25%(v/v) glycerol, 0.1M dithiothreitol, 1mM bromophenol blue, pH6.8) and heated for 5mins at 100°C. The proteins contained in a 10µl aliquot of each sample were then separated according to their mass, using the SDS-PAGE system described by Laemmlli (1970). Electrophoresis was performed in 15% gels at 150v for 1h. Upon completion of SDS-PAGE, the gel was equilibrated in western blot transfer buffer (25mM tris/HC1, 192mM glycine, 20%(v/v) methanol) for 30mins. Proteins were then transferred from the gel to a nitrocellulose membrane by electroblotthing at 30v overnight. The nitrocellulose membrane was incubated at room temperature with a phosphate buffered saline/Tween-20 solution (PBS-Tween) for 30mins and then incubated for a further 60mins in a solution of rabbit-anti-MUP serum (RαMUP), diluted 1:5000 with PBS-Tween. Following this, the membrane was washed three times in PBS-Tween prior to being incubated at room temperature for a further 60mins in a solution of goat-anti-rabbit alkaline phosphatase conjugated antibodies (purchased from Bio-Rad), diluted 1:3000 with PBS-Tween. The membranes were again washed three times with PBS-Tween and subsequently developed with a solution of 0.15mg/ml 5-bromo-4-chloro-3-indoyl phosphate (BCIP), 0.3mg/ml nitro blue tetrazolium (NBT) in 0.1M tris/HC1 buffer, pH9.5 containing 5mM $MgC1_2$.

2.3 Assessment of the MUP status of wild mouse urine

To confirm the presence of MUPs in urine from wild mice, the urine of 17 wild caught mice, 10 males (MU1, 2, 3, and 6, M2, M3, A, B, C and F) and seven females (FU1-7), along with urine from BALB/c inbred mice, was subjected to western blot analysis with RαMUP serum. Urine from these wild mice was collected

reclaimed by overlaying the dried urine with three 200µl aliquots of deionised water. The aliquots were pooled and their volume reduced to 100µl by rotary evaporation. A 50µl aliquot of this solution was then removed and an equal volume of SDS-PAGE sample buffer was then added to it prior to heating at 100°C for 5mins. Western blot analysis was then performed on such samples using the protocol outlined above.

2.4 Visualisation of urine deposits transferred onto a nitrocellulose membrane

A clean Perspex tile, 15cm x 15cm, was placed into the enclosure of a captive, wild caught mouse for 30mins. It was then removed and allowed to dry. The tile was then overlaid with a nitrocellulose membrane of the same dimensions. This was then wetted by overlaying with a sponge soaked in deionised water which was firmly rolled against the membrane. The whole apparatus was then weighted down and left for 60mins. Urine deposits bound to the membrane were then visualised using the protocol described above. The developed membrane was subsequently scanned into a computer and mapped to a false colour image.

2.5 Visualisation of urine deposited directly onto a nitrocellulose membrane

To avoid problems caused by mouse chewing, a nylon backed nitrocellulose membrane was used to collect urine deposits directly from the mouse enclosure. This combined the protein immobilising properties of conventional nitrocellulose with great mechanical strength. Two pieces of such membrane, each 9 x 15cm, were used to line a Perspex tunnel attached to the entrance of a nest box in two enclosures housing two individual, wild caught, male house mice. The membranes were left in this position for 30mins prior to being removed and allowed to dry. The urine marks deposited as the mice passed through the tunnels were then visualised using the protocol described above.

2.6 Determination of immunodetection technique sensitivity

Urine was collected from six adult, male BALB/c mice and pooled. The protein concentration of the pool (25mg/ml) was measured using the Coomassie Plus

microtitre [illegible] The urine was titrated [illegible] solutions with a range of protein concentrations (10, 5, 2 and 1µg /ml; 100, 10 a[illegible] 1ng/ml). A 1µl aliquot of each solution, in addition to the neat urine, was spotted onto a nitrocellulose membrane. The membrane was allowed to dry prior to being developed in the manner outlined above.

3. RESULTS

3.1 Confirmation of the presence and specificity of anti MUP antibodies in immunised rabbit serum

Figure 1 illustrates that Western blot analysis of mouse urine, serum and liver homogenate gives a single band in all three samples. In the experiments illustrated in figure 1, urine from BALB/c mice, mouse serum and a mouse liver homogenate were all analysed by western blot. Urine was diluted 1:500, serum 1:20 and liver homogenate 1:10. Two 10µl aliquots of each sample thus prepared were loaded onto the gel. After transfer to a nitrocellulose membrane, the samples were incubated with rabbit-anti-MUP serum, followed by a solution of goat-anti-rabbit antibodies conjugated to alkaline phosphatase. The membrane was then developed with a solution of 5-bromo-4-chloro-3-indolyl phosphate and nitro-blue tetrazolium.

The MUP concentration in urine in laboratory mice has been estimated as 15mg/ml. Therefore the 1:500 dilution of urine analysed in Figure 1, has an estimated MUP concentration of 30µg/ml. The 10µl aliquot of this solution loaded onto the gel contains an estimated 300ng of MUP, demonstrating the sensitivity of the technique. The serum and liver homogenate samples each contain large numbers of other proteins. The presence of a single band after western blot analysis of these two samples indicates that the anti-MUP antibodies in the rabbit serum react specifically with MUPs.

3.2 Assessment of MUP status in wild mouse urine

By virtue of their immunoreactivity and electrophoretic mobility in the western blot analysis, MUPs were identified in the urine of seventeen mice (see Fig. 2).

and females (FU1, 2, 3, 4, 5, 6 and 7), were supplied as dried deposits on microscope slides. Each sample was overlaid with 3 x 200ml of de-ionised water. The pooled aliquots were dried down to approximately 100ml in a rotary evaporator. Proteins in these preparations were separated by SDS-PAGE prior to being electroblotted onto a nitrocellulose membrane. This membrane was subsequently probed with antiserum raised against BALB/c MUPs. A control of BALB/c MUPs was run on each gel to allow comparison of molecular mass.

The concentration of MUPs was generally greater in the male urine confirming the sexual dimorphism of MUP expression. The samples with the lowest concentration still gave a visible reaction to this type of analysis. As all these samples were dried prior to analysis, the results indicate that the deposition process does not significantly affect the immunoreactivity of such MUPs to the RαMUP antibodies.

3.3 Visualisation of urine deposits transferred to nitrocellulose membrane

Urine deposits transferred from a Perspex tile onto a nitrocellulose membrane were readily visualised using immunostaining (see Fig. 3).

In Figure 3, a perspex tile (15cm x 15cm) was placed in a captive, wild mouse enclosure for 30 min. The tile was then removed and allowed to dry. The marked face of the tile was then overlaid with a nitrocellulose membrane of the same dimensions. This was then wetted by overlaying with a sponge soaked in distilled water, and firmly rolled against the membrane. The whole assembly was weighted down and left for 1h. Membrane bound proteins were visualised by probing firstly with a rabbit anti MUP antiserum (raised against MUPs from inbred mice), followed by a goat anti rabbit-alkaline phosphatase conjugate. Visualisation of such antibody complexes was achieved using a NBT/BCIP substrate. The developed membrane was scanned into a computer and mapped to a false colour image.

As can be seen from Figure 3, in addition to the streaks and pools of urine deposited by the mouse, there are a large number of small dots. These are thought to

deposition of MUPs and the sensitivity of this detection system, have obvious applications in the detection of low level rodent infestations.

Visualisation of MUPs as described above represents a preferred method of detecting for rodent infestations according to the method of the invention.

3.4 Detection of urine deposited directly onto a nitrocellulose membrane

Successful visualisation of urine deposits directly immobilised onto nitrocellulose has also been possible using RαMUP antibodies (see Fig. 4).

For the data presented in figure 4, two pieces of nylon backed nitrocellulose membrane, 9 x 15cm were placed inside Perspex tunnels which were subsequently placed between the nesting box and enclosure of two wild mice, A and H. The tunnels were left in this position for 30min. The nitrocellulose membrane was then removed and incubated with a buffered solution of rabbit antiserum raised to MUPs from inbred mice. Visualisation of bound antibody was achieved by a further incubation with a goat anti-rabbit-alkaline phosphatase conjugate solution followed by incubation with a NBT/BCIP substrate solution.

As can be seen from Figure 4, the two membranes placed in the nest box-enclosure tunnels both gave positive reactions. The two membranes show slightly different patterns of deposition. The urine deposited by mouse H seems to be in the form of footprints, whilst mouse A also shows more obvious signs of direct urine deposition (streaks and pools). This visualisation of urinary proteins carried solely on the paws of the mouse once again attests to the sensitivity and applicability of the method of the present invention.

3.5 Sensitivity of detection assessment

The sensitivity of the immunoassay was assessed by testing various concentrations of mouse urine.

25mg/ml using a Coomassie dye binding assay. The urine was then diluted to give concentrations of 10, 5, 2 and 1µg/ml and 100, 10 and 1ng/ml. A 1µl aliquot of each solution was then placed on a nitrocellulose membrane and allowed to dry. The membrane was subsequently developed with rabbit-anti-MUP serum followed by goat-anti-rabbit antibodies conjugated to alkaline phosphatase. Immunoreactive protein was then visualised by treating the membrane with a solution of 5-bromo-4chloro-3-indolyl phosphate and nitro blue tetrazolium.

Figure 5 illustrates that the immunodetection technique used according to this Example detected 1ng of MUPs deposited directly onto a nitrocellulose membrane.

In assessing the level of detection, no attempt was made to optimise the conditions under which immunodetection was performed. Such optimisation could further increase the sensitivity of detection. Greater sensitivity could also be achieved using a chemiluminescent detection system as opposed to the chromogenic detection system used in this instance.

4. SUMMARY

Detection of rodent infestations according to the method of the invention and particularly as described in this Example (which represents a preferred embodiment of the invention) represents a much more sensitive detection method than previously known in the art..

The data presented here demonstrate that antibodies raised in a rabbit, in response to urinary proteins from laboratory mice, can be used to specifically and sensitively detect urine deposits from wild mice according to the method of the invention. The sensitivity of this technique is such that it has detected minute quantities of MUPs carried on the feet of mice. This unwitting form of deposition will allow detection of low level rodent infestation and identification of the precise sites being used. This will, in turn, allow efficient targeting of pest control measures.

The experiments described above were all performed using mouse urine. However it will be appreciated that the presence of α2u globulins in rat urine allows the same principles to be applied to rat urine.

The possibility also exists to discriminate between the two species when investigating a potential infestation. Three other rodents, the black rat (*Rattus rattus*) the bandicoot rat (*Bandicota bengalensis*) and the multimammate mouse (*Mastomys natalensis*) also live in close proximity to human populations (largely in Asia) and we envisage a similar method of detection may be applied for the detection of infestations of these species.

1. A method of detecting a rodent infestation comprising detecting for the presence of urinary proteins from rodents on a surface on which urinary proteins have been deposited.

2. The method according to claim 1 wherein the urinary proteins are Major Urinary Proteins from mice.

3. The method according to claim 2 wherein the major urinary protein has an amino acid sequence as defined by Genbank Accession Numbers X00907, M16355, M16356 or X00908 and functional derivatives thereof.

4. The method according to claim 1 wherein the urinary proteins are α2u globulins from rats.

5. The method according to any preceding claim wherein the presence of urinary proteins from rodents is detected by utilising antibodies raised against the urinary protein in an immunoassay.

6. The method according to claim 5 wherein the antibodies comprise primary antibodies which binds to the urinary protein and secondary antibodies which are associated with a detectable signal and also binds to the primary antibody.

7. The method according to any preceding claim wherein swab samples are taken from the surface and the presence of urinary proteins detected on the swabs.

8. The method according to any one of claims 1-6 wherein a nitrocellulose membrane is laid on the surface and the presence of urinary proteins detected on the membrane.

over the surface and the presence of urinary proteins detected on the substratum.

10. The method according to claim 9 wherein the substratum is a sheet or tile.

11. The method according to claim 9 or 10 wherein a nitrocellulose membrane is laid over the tile or sheet and the presence of urinary proteins detected on the membrane.

12. A method of detecting a rodent infestation comprising detecting for the presence of urinary proteins from rodents on a surface over which a rodent has putatively travelled.

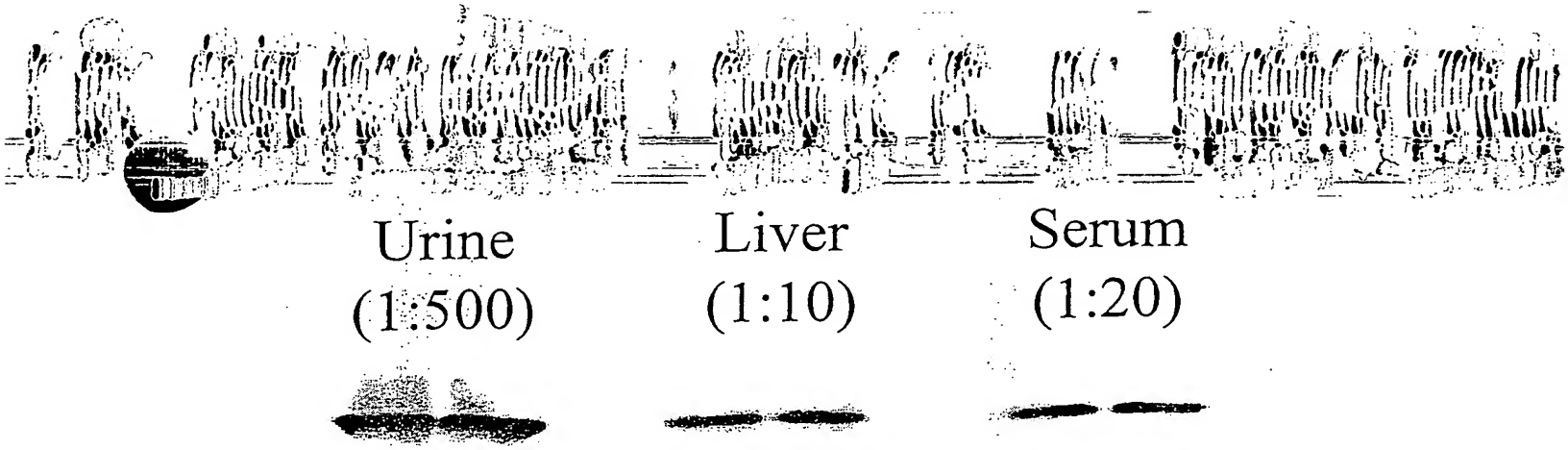

Urine
(1:500)
Liver
(1:10)
Serum
(1:20)

Fig. 1

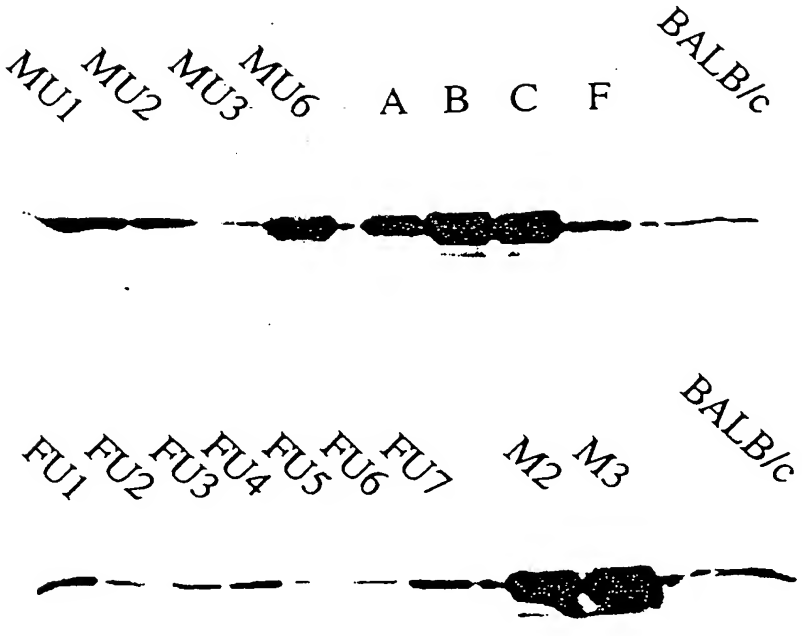
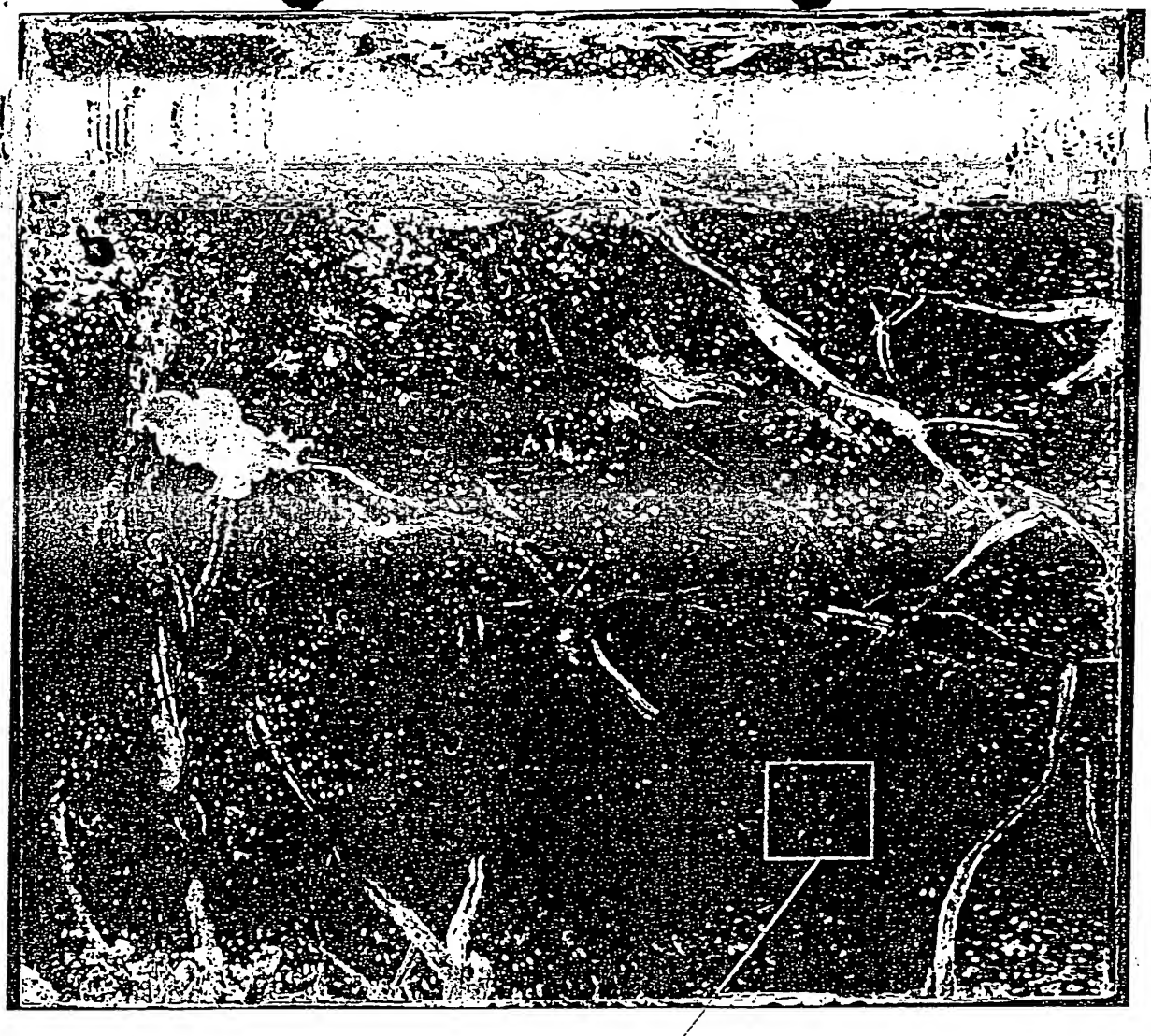

MU1
MU2
MU3
MU6
A
B
C
F
BALB/c
FU1
FU2
FU3
FU4
FU5
FU6
FU7
M2
M3
BALB/c

Fig. 2



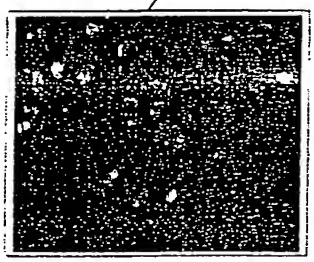
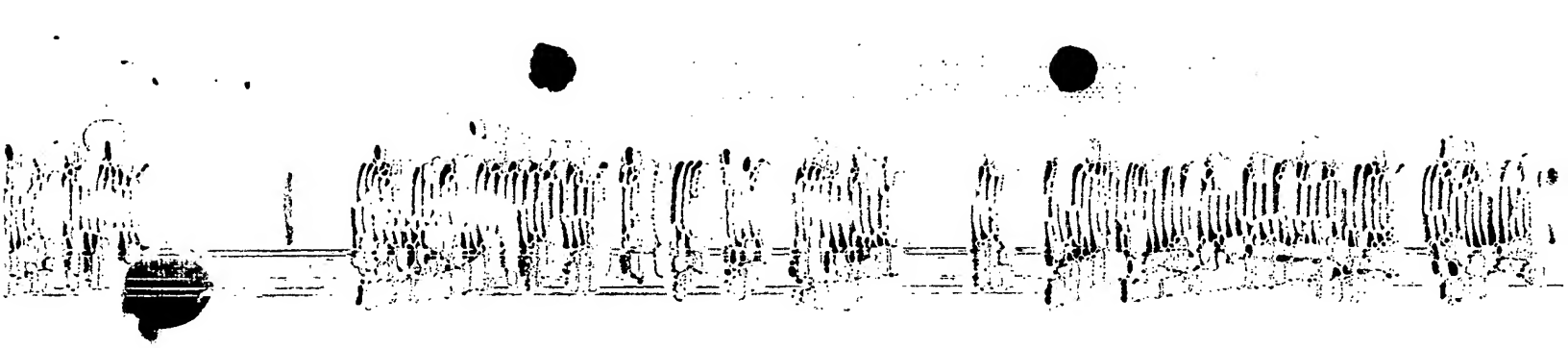


Fig. 3



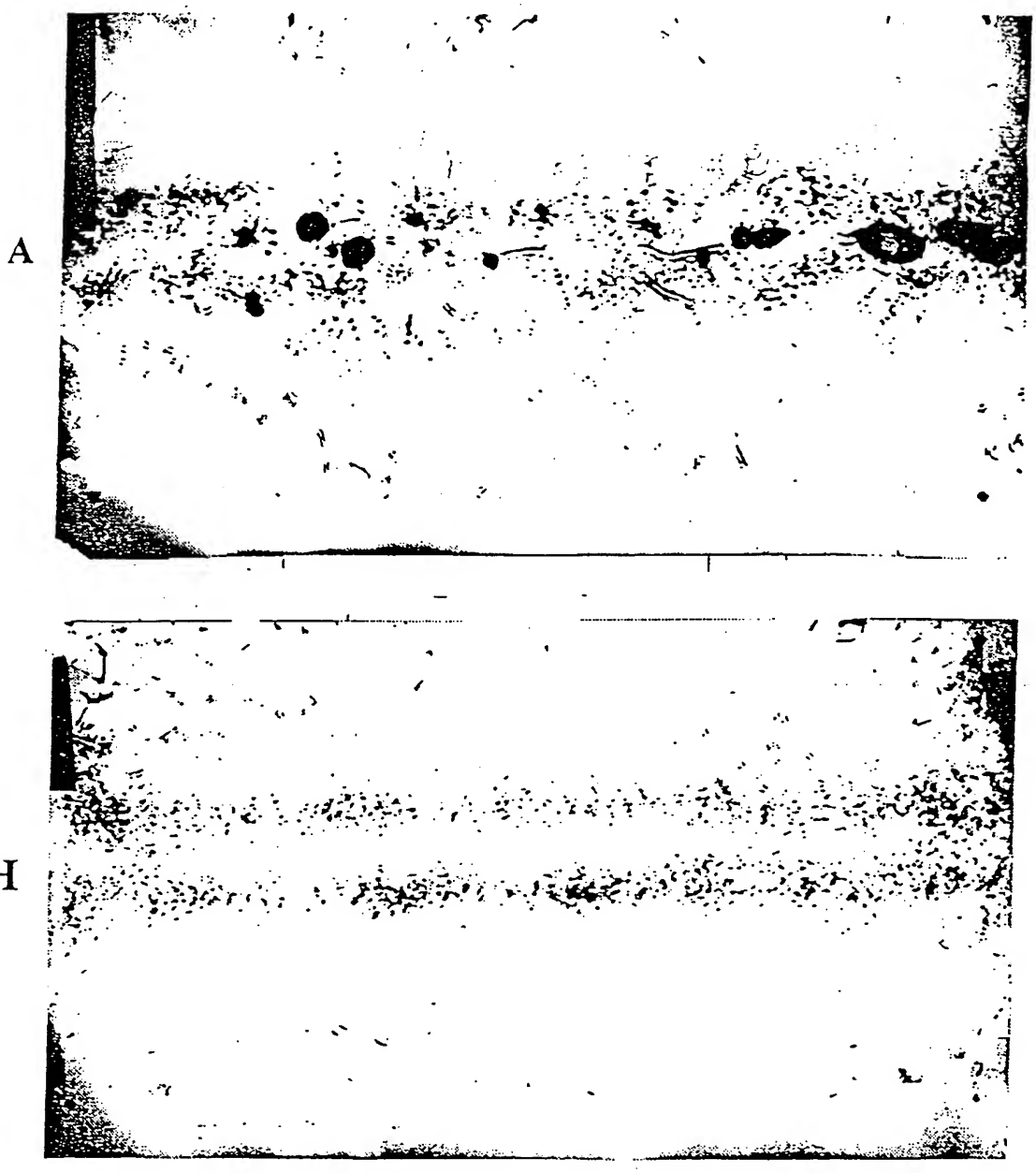


Fig. 4

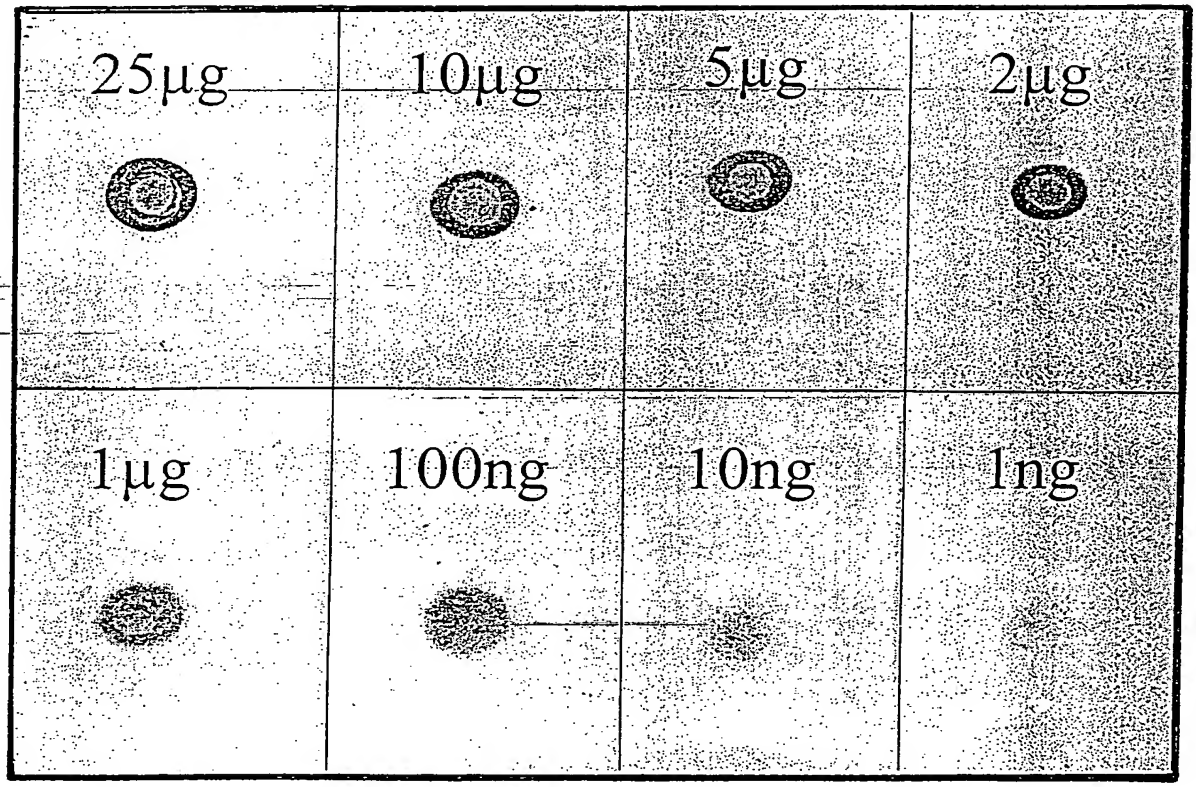


FIG.5